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SEXUALITY IN RHIZINA UNDULATA FRIES

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(WITH PLATES III AND IV)

Although in recent years a considerable number of papers have appeared dealing with the phenomena of sexuality in the Ascomycetes, certain of the natural orders in this group have received little attention. In the Discomycetes practically all the species which have been investigated are members of the Pezizales, and few facts are available concerning the sexual process in representatives of any other order. Our knowledge of the morphology of the sexual organs and the behavior of the sex nuclei in the Helvellales is particularly meager. The following brief discussion of the important papers which have been published on the cytology of these fungi will serve to emphasize this fact.

BROWN (11) describes the development of the ascocarp in two species of *Leotia*. In *L. lubrica*, at the base of the youngest fruit-body sectioned, he discovered a large, vacuolated cell having the appearance of an emptied ascogonium. From this cell he found arising a number of hyphae of larger diameter than the other hyphae of the ascocarp. These larger hyphae were empty, and, although they could not be followed for any great distance, they seemed to be connected higher in the fruit-body with the ascogenous hyphae. He apparently found evidences of this ascogonium-like structure in only one specimen, since he states nothing to the contrary, and does not describe other stages in its development. He gives no data

concerning nuclear conditions in this cell nor in the hyphae to which it gives rise, and makes no mention either of the presence or absence of an antheridium. Moreover, in the single other species which he studied, *L. chlorocephala*, he finds no indication of the presence of sexual organs, and states merely that the ascogenous hyphae have their origin in the stipe.

CARRUTHERS (14) discusses in some detail the cytology of *Helvella crispa*, placing particular emphasis on the nuclear divisions in the ascus. He states definitely that sexual organs are absent in this species, and describes apogamous nuclear fusions in undifferentiated hyphae of the hypothecium. Although in the summary of his paper he states that the cells containing the fusion nuclei give rise to the ascogenous hyphae, this important point is not mentioned in the discussion of his results and no figures are given demonstrating it. He states further that "there is evidence that mitoses in the vegetative and ascogenous hyphae show respectively 2 and 4 chromosomes," and says that the third nuclear division in the ascus is brachymeiotic, there being 4 chromosomes in the prophase, while only 2 pass to the poles.

DITTRICH (24) states that in *Mitrula phalloides* the ascogenous hyphae arise near the center of the fruit-body from a complex of closely massed, elongated, deeply staining filaments characterized by the possession of large nuclei with prominent nucleoli. He finds no sexual organs, and does not describe an approximation or fusion of nuclei in the hypothecium.

McCUBBIN (53) states that in *Helvella elastica* "no structure having the conventional form of an ascogonium" is found, and says that the ascogenous hyphae "arise as a clearly differentiated subhymenial complex of filaments." However, he describes at considerable length large cellular bodies which occur irregularly throughout the whole of the ascocarp except the stem. He regards these as vegetative in function and calls them storage bodies. In the earliest stages in the development of the fruit-body they are absent, but they appear relatively early. They are large, attaining in some cases 20–30 times the diameter of the surrounding hyphae, are filled with deeply staining protoplasm, and exhibit remarkable variation in shape. They sometimes form a chain

of 3 or 4 connected cells. By the time the asci are mature they are usually empty, their connections have disappeared, and their walls have collapsed. McCubbin states that these structures in some instances give rise to palisade hyphae and paraphyses, while at other times they are found "having the ascogenous hyphae proceeding directly from them." They contain nuclei varying in number from 1 to 20 or more, a conspicuous feature being the frequent arrangement of these nuclei in pairs. McCubbin gives a number of illustrations showing the variation in size and shape of these structures and demonstrating clearly the paired condition of the nuclei. Several significant facts would seem to indicate that at least part of these "storage bodies" constitute some type of sexual apparatus, particularly the statement that they are sometimes found giving rise to the ascogenous hyphae.

Faull (**26**) discusses the method of origin of the asci from the ascogenous hyphae in a considerable number of species representative of various genera of the Helvellales. He has not investigated the sexual process, however, or described sexual organs in any of the forms studied.

In so far as the writer is aware, no other papers of importance bearing on the sexual process in the Helvellales have appeared. In no member of this group is our knowledge more than fragmentary; in fact it cannot be stated with certainty that any worker has seen the sexual organs in any species of the order. The family Rhizinaceae has received no attention whatever from the standpoint of cytology. As representative of this family, *Rhizina undulata* Fries, is especially suitable for investigation. It is the type of the genus and the family, and probably the most widely distributed and best known member of the group.

Materials and methods

In the summer of 1914 the writer collected a considerable number of apothecia of *Rhizina undulata* in a small pine wood north of Beebe Lake near the Cornell University Campus at Ithaca, New York. Fruit-bodies of practically all stages of development were obtained. The youngest stages, including undifferentiated primordia, were studied, and the results of the investigation were

embodied in an account of the origin of the ascocarp in this species (FITZPATRICK 27). During the course of this investigation the examination of certain slides disclosed the fact that the material was favorable for a study of the sexual process. Additional slides were then prepared, and material of all ages was given critical examination.

The apothecia were fixed in the field in medium strength chromo-acetic acid fixer, and were later imbedded in paraffin. The material was studied in serial sections 4–7 μ in thickness and was stained in most cases with Haidenhain's iron alum-haematoxylin, although for certain stages the shortened Flemming's triple stain proved more satisfactory.

Certain of the apothecia on which the investigation is based were sectioned and stained in the laboratories of the Brooklyn Botanic Garden in the summer of 1915, while the writer held a visiting fellowship at that institution. He wishes to express here his appreciation of the courtesy of Director C. S. GAGER in extending to him all the facilities of the laboratories and gardens, and to acknowledge his indebtedness to Dr. E. W. OLIVE for many kindnesses, including helpful suggestions concerning microtechnique. Subsequently other apothecia were sectioned and stained in the laboratories of the Department of Plant Pathology at Cornell University. All the critical study of the material was made at the latter institution during the spring of 1916. The writer's identification of the species as *Rhizina undulata* was confirmed independently by Dr. E. J. DURAND and Dr. F. J. SEAVER. The completed manuscript was examined by Professor GEORGE F. ATKINSON. His criticisms, especially with reference to the interpretation of the meaning of the paired condition of the nuclei in the cells of the archicarp, have been embodied in the text, and have resulted in extensive alterations. The writer wishes to express his appreciation of these favors.

Vegetative hyphae

The mycelium of *Rhizina undulata* is parasitic on the roots of various trees (HARTIG 42, 43, 44, TUBEUF 63, WEIR 64). It develops profusely in the soil also, enveloping the soil particles and smaller roots as a whitish, moldlike growth. On the surface of the ground

and on partially exposed roots a definite subiculum is thus produced, upon which minute, snow white knobs of mycelium are developed. These constitute primordia of fruit-bodies. They are composed of undifferentiated hyphae, but a somewhat indefinite palisade layer is formed over the periphery of the primordium. At their initiation, these primordia are extremely minute, averaging approximately 0.3 mm. in lateral diameter. There is no indication other than shape that they are to develop into ascocarps. Sexual cells at this early period are certainly absent. The hyphae composing the primordium are all of approximately the same diameter, and consist of narrow, cylindrical, multinucleate cells. Uninucleate or binucleate cells are not found. These hyphae in many instances can be traced back toward the point of origin of the primordium, where they are either lost in the tangle of hyphae composing the subiculum or are found to enter the soil.

The ascocarp primordium increases in size chiefly by the elongation and branching of the palisade hyphae at the periphery. At the same time the palisade layer becomes more sharply demarcated. The fruit-body, as demonstrated by the writer in his earlier paper, is not, either at the beginning or at any later period, provided with an enveloping membrane. The ascocarp in this species is therefore gymnocarpous, the hymenium being "exposed from the first."

The nuclei in the cells of the vegetative hyphae are small, and were studied with difficulty. A small amount of chromatic material and a deeply staining nucleolus may be seen in each. No division figures have been observed. It is possible that mitosis occurs only at night, all the material having been placed in the fixer at one time during the day. However, the minute size of the nuclei would render any study of nuclear division in the vegetative hyphae extremely difficult. The nuclei occur irregularly throughout the hyphae, and give no indication of pairing or of any other definite arrangement. Deeply staining granules are present in the cytoplasm. These extranuclear bodies, possibly the metachromatic granules of GUILLIERMOND (35), are of doubtful function. Sometimes they are found grouped over the opposite faces of the transverse septa. A similar condition exists in *Ascophanus carneus*, where, according to CUTTING (18), they guard a minute pore in the

septum. In *R. undulata* no such pores have been demonstrated. Such protoplasmic connections, however, are of frequent occurrence in the fungi. They were first observed by CHMIELEWSKY (15). Subsequently they have been the object of research by DANGEARD (19, 20, 21) in *Sphaerotheca Humuli*, *Bactridium flavum*, and other fungi; and have been studied in various species by MASSEE (52), KIENITZ-GERLOFF (48), MEYER (54), GUILLIERMOND (37), and others. MEYER in particular has given them considerable attention and has demonstrated that open pores exist in the transverse septa of the hyphae of many Basidiomycetes and Ascomycetes. They possibly function in permitting a more rapid transfer of food material from cell to cell.

Archicarp

When the ascocarp primordium has attained a diameter of approximately 1 mm., differentiation begins to take place, certain hyphae lying near its center undergoing transformation into archicarps. The number of archicarps developed in the interior of a single ascocarp varies, and when several archicarps lie closely approximated their interweaving renders an exact count difficult. A careful study, however, of all the consecutive sections of a complete series through the ascocarp demonstrates that the number is in some cases as many as 8, and in many individuals probably more. No ascocarp containing less than 3 archicarps has been found. Although lateral fusion of adjacent apothecia resulting in the formation of irregular compound structures is a common phenomenon, it fails to explain the presence of more than a single archicarp in a fruit-body. Ascocarps of circular form which are clearly the result of the enlargement of a single primordium contain several archicarps. Moreover, young primordia in which lateral fusions have certainly not taken place reveal several archicarps in the process of development.

While the production of several archicarps in a single apothecium is unusual in the Discomycetes, this condition being more typical of the discomycetous lichens, it is not unique. OVERTON (56) finds that in *Thecotheus Pelletieri* the apothecium is compound, the fruit-body arising from several multicellular archicarps. In other Discomycetes, of which *Pyronema confluens* is perhaps the

best known example, several pairs of ascogonia and antheridia contribute to the formation of a single apothecium. In the majority of the Discomycetes which have been studied, however, a single archicarp is developed. Of these may be enumerated *Lachnea scutellata* (BROWN 12, WORONIN 69), *Peziza granulosa* and *Ascobolus pulcherrimus* (WORONIN 69), *Ascobolus furfuraceus* (JANCZEWSKI 45, 46, HARPER 39, WELSFORD 65), *Ascodesmis nigricans* (VAN TIEGHEM 61), *Ryparobius* sp. (BARKER 4, 5), *Thelebolus stercoreus* (RAMLOW 57), *Lachnea scutellata* (BROWN 12), *Humaria granulata* (BLACKMAN and FRASER 9), *Ascophanus carneus* (CUTTING 18), and *Lachnea cretea* (FRASER 30). As representative of lichens containing several archicarps in a single apothecium may be listed *Parmelia acetabulum* (BAUR 7, 8), *Anaptychia ciliaris*, *Lecanora subfusca*, *Endocarpon miniatum*, *Gyrophora cylindrica*, and *Cladonia pyxidata* (BAUR 8), *Pertusaria communis* and *Pyrenula nitida* (BAUR 7), and several species of *Collema* (BAUR 6, BACHMANN 2, 3).

The individual archicarp of *R. undulata* arises by the rapid growth and transformation of a single multicellular hypha. The cells increase greatly in lateral diameter and become filled with deeply staining protoplasm, so that the resulting structure assumes a dense and opaque appearance. The relatively few nuclei originally present undergo repeated division, and each cell of the archicarp is soon packed with many nuclei. The cells of the archicarp are certainly multinucleate from the first. In *Ascobolus*, according to HARPER (39) and WELSFORD (65), the cells of the archicarp are uninucleate at the beginning, while in other forms (BROWN 12 *Lachnea scutellata*, CUTTING 18 *Ascophanus carneus*) they are described as multinucleate in all stages.

The diameter of the cells of the archicarp when the ultimate size is reached is much greater than that of the surrounding hyphae, and for this reason no possibility exists of mistaking an archicarp for an ordinary hypha, even when the lower powers of the microscope are used. This difference in size is strikingly shown in fig. 4. Cells of a mature archicarp sometimes measure 10 times the diameter of the other hyphae.

The archicarp is in all cases multicellular, the number of cells varying in the counts made from 10 to 19. Different individuals

have been followed carefully from base to apex throughout the various sections of a series, and the cells are found to differ to a marked degree in size and shape. Great variability is also shown in the general form of the archicarp (figs. 1–4). It develops in some cases as a loose coil (fig. 3), in others winds irregularly among the other hyphae (fig. 1), or more rarely bends back upon itself, forming two nearly parallel rows of cells (fig. 4). Irregularly winding archicarps are the most common type. Closely wound coils have not been found. Antheridia are not produced, and no fusion of the terminal cell of the archicarp with any other structure has been observed. Many sections have been examined in vain in an endeavor to demonstrate such fusions. The writer is convinced that none occur.

The terminal cell of the archicarp is smaller than the other cells of this structure. It is usually narrow and attenuated, and at the maturity of the archicarp shows disorganized, deeply staining, protoplasmic contents. It resembles very closely the cell figured and described by CUTTING (18) as a trichogyne in *Ascophanus carneus*, and from analogy the writer will refer to it as the trichogyne. It certainly does not function, however, and is evidently merely a vestigial structure.

The archicarp in *R. undulata* is not, as in certain other species, sharply divided into definite apical, central, and basal portions. The cells which give rise to ascogenous hyphae are usually centrally located in the coil, and in some individuals are slightly larger than the other cells, but this is not always the case. No well defined ascogonium is differentiated.

In the younger stages in the development of the archicarp no pores can be detected in the transverse septa. If any exist, they are very minute. Deeply staining, extranuclear granules, resembling those in the vegetative hyphae, are frequently found grouped on opposite sides of the cross walls. Their occurrence is not constant and their function is unknown. Similar granules are also described as occurring in *Ascobolus* (HARPER 39, WELSFORD 65), *Ascophanus carneus* (CUTTING 18), *Pyronema confluens* (HARPER 40), *Humaria granulata* (BLACKMAN and FRASER 9), and other Ascomycetes.

As the archicarp of *R. undulata* approaches maturity a very prominent, deeply staining, hemispherical or convex pad appears on each side of each cross wall at or near its center. Similar pads have been found in *Humaria granulata* (BLACKMAN and FRASER **9**), *Ascophanus carneus* (CUTTING **18**), and other forms, but in no case have the figures presented by the investigator shown such striking and definite structures as those in *R. undulata*. Since at a somewhat later period a single large pore appears in each of the transverse septa at the point earlier occupied by the pads, it seems probable that the latter represent a swelling out of the septum due to gelatinization at this point. CUTTING has suggested that the metachromatic granules mentioned may function in bringing about such a gelatinization. It is certain, in any case, that the pads are absent in young archicarps; that with the approach of maturity they are prominent; and that still later they disappear, leaving behind a well defined pore in the septum. CUTTING found pads in *Ascophanus carneus* lying free in the cytoplasm of the archicarp following the appearance of the pores. Attached to these he observed what seemed to be bits of the wall on which they originally lay. The writer, however, has not seen any such detached pads in *R. undulata*.

Near the apex of the archicarp shown in fig. 3 may be seen the union of the two pads which originally lay separated on the opposite faces of the septum. We may assume that this fusion represents the last stage in gelatinization. CUTTING (**18**) figures a similar condition (his fig. 14) in an archicarp of *Ascophanus carneus*.

Although the disappearance of these pads takes place suddenly, the process does not occur simultaneously on all the transverse septa. In fact, neither in the development of the pads nor in their removal is any definite sequence followed as regards the relative position of the septa in the archicarp. In the youngest archicarp shown (fig. 1) not all of the pads have been formed. In an older archicarp (fig. 4) all have disappeared, leaving definite open protoplasmic connections. In intermediate stages (figs. 2, 3) some pads have disappeared while others remain.[1] Rarely a single pair of pads persists on a septum until the formation of ascogenous hyphae has

[1] Read the introductory paragraph in the explanation of plates.

progressed to a marked degree (fig. 7). The mature archicarp, on account of its very dense protoplasmic contents and numerous nuclei, stains very deeply, and in many cases is practically opaque. Not all the individuals stained prove favorable, therefore, for the demonstration of protoplasmic continuity. Moreover, on account of the winding course of the archicarp, which results in the appearance of different portions of a single coil in several different sections, not all of the pores or pads are visible in the plane of one section. When the position of the archicarp is favorable careful staining renders the pores very evident (figs. 4, 7). They are slightly greater in diameter than a single nucleus. The ascogenous hyphae in some cases (figs. 3, 7) arise before all of the pads have disappeared; in other cases (fig. 4) all of the pores may be formed before any indication of the development of ascogenous hyphae is given.

Ascogenous hyphae

As stated earlier, no definite group of cells in the archicarp gives rise to the ascogenous hyphae. Usually 4 or 5 consecutive cells lying near the center of the coil function as ascogonial cells. These put out a considerable number of ascogenous hyphae, which by repeated branching develop a large number of free ends for the formation of ascus hooks. The other cells of the archicarp meantime fail to bud, and their nuclei and cytoplasm flow through the open connections in the transverse septa into the active ascogonial cells and thence into the ascogenous hyphae. All the cells of both the apical (exclusive of the trichogyne) and basal regions contribute their contents to this general flow, and are finally almost entirely emptied. This migration is shown clearly in figs. 5 and 6. Figs. 8, 9, 10, and 11 represent at a considerably higher magnification sections through ascogonial cells at right angles to the long axis of the archicarp. In two of these (figs. 10, 11) the ascogenous hyphae are shown at their point of origin from the archicarp. The others (figs. 8, 9) represent sections through budding cells at points between the places where hyphae arise. A pronounced vacuolation of the cytoplasm of the ascogonial cells occurs at the time of the outward flow of nuclei into the ascogenous hyphae. Since the vacuolation is more evident in the center of the cell, the nuclei

which remain behind lie at this stage in a rather restricted zone at the periphery.

This pronounced vacuolation and thinning of the cytoplasm of the ascogonial cells renders less difficult the study of the nuclei, and at this stage, in the writer's preparations, they seem always to lie in pairs. At no other stage in the development of the archicarp, either before or after the formation of pores in the transverse septa, have paired nuclei been found in any of the cells of this structure. This, however, may be due in large measure to the fact that the dense nature of the cytoplasm and the crowding of the nuclei render the determination of this point extremely difficult.

The presence of paired nuclei in any of the cells of the archicarp is a matter of the greatest interest and importance. This is especially true since an antheridium is absent. The determination of the origin of the two nuclei which constitute a pair, however, is fraught with considerable difficulty. It is evident that they are either potential sex nuclei which have had their respective origins in the same or different cells of the archicarp, or sister nuclei which have resulted from a recent more or less simultaneous division of the archicarp nuclei. If they are sex nuclei, it is to be expected that they will either fuse in the archicarp or migrate side by side into the ascogenous hyphae, where they will undergo conjugate divisions preceding the fusion in the ascus.

Fusion of these pairs of nuclei in the cells of the archicarp has not been observed. Although occasionally the two nuclei lie in actual contact, fusion stages have not been found. Moreover, no nuclei of larger size have been seen which might from analogy be assumed to be fusion nuclei. A thorough examination of the nuclei in the ascogenous hyphae has failed, moreover, to demonstrate conjugate divisions. In some instances groups of nuclei in fours have been found lying in such a position as to suggest their origin from conjugate divisions, but these cases are not numerous enough to carry conviction. No mitotic figures, either of simple or conjugate division, have been seen in these hyphae, nor in any of the cells of the archicarp. The writer has attributed their absence to the fact that all of his material was placed in the fixing solution at one time. Periodicity of mitosis thus could easily explain their

absence in all of the preparations. Since *R. undulata* is an uncommon species, it is infrequently collected, and the writer, desirous of supplementing his material with preparations showing mitosis, searched for the fungus without success throughout the summers of 1915 and 1916. While unwilling to state that conjugate divisions do not take place in the ascogenous hyphae of this species, he has been unable to demonstrate their occurrence. On the other hand, a periodicity in mitosis which would constitute a more or less simultaneous division of all the nuclei in the archicarp might easily give at the rounding up of the daughter nuclei a marked appearance of pairing. The pairs of nuclei in the ascogenous hyphae could also originate in the same manner. Until mitotic figures, either of simple or conjugate divisions, have been demonstrated in the ascogenous hyphae, it will be well to reserve judgment as to the meaning of the paired condition.

A comparison of our work on *R. undulata* with that of other investigators who have studied the origin of the paired condition in those Ascomycetes in which a male organ is lacking or nonfunctional is not enlightening. Although great variation exists in their accounts, fusion of nuclei in pairs in the ascogonial cells is described as occurring in *Lachnea cretea* (FRASER 30), *Ascophanus carneus* (CUTTING 18), and *Thecotheus Pelletieri* (OVERTON 56). Conjugate divisions have not been described in any case. CLAUSSEN (17) alone in *Pyronema confluens* has figured conjugate divisions in the undifferentiated portions of the ascogenous hyphae of the Discomycetes.

The ascogenous hyphae of *R. undulata* undergo repeated branching as they approach the hymenium. They soon become multiseptate (fig. 12), the individual cells containing a varying number of nuclei which are usually, though not constantly, in evident pairs. On the transverse septa are found deeply staining granules resembling those in the vegetative hyphae. In some cases these are aggregated into large granules similar to the deeply staining pads of the archicarp, but they are in reality much smaller. Other granules occur throughout the cytoplasm. No open pores in the septa have been demonstrated, and although it is possible that minute protoplasmic connections exist, there is no reason to think that nuclei migrate from cell to cell. In later stages the deeper lying

cells of the ascogenous hyphae become vacuolated, stain lightly, and apparently take no direct part in the formation of the hymenium.

The layer of paraphyses is developed early in the history of the fruit-body and constitutes a well defined zone long before the young asci are developed. This zone is in reality merely a differentiation of the palisade layer of peripheral vegetative hyphae, and its elements have no direct organic connection with the archicarp or ascogenous hyphae.

Early in the history of the archicarp there are developed also paraphysis-like structures, termed setae, which originate far below the hymenium from vegetative hyphae, traverse the hymenium, and protrude beyond it as thick-walled, dark-colored spines. These spines are non-septate tubes which discharge at their tips a brown, glutinous secretion over the surface of the hymenium.

The terminal branches of the ascogenous hyphae push up to the base of the paraphysis layer, and there undergo typical crozier formation. The terminal portions of the hyphae are of smaller diameter than the cells nearer the archicarp. The tip of each branch contains two nuclei, and in some cases these are cut off from the remainder of the thread by a septum. The nuclear membrane is sharply defined and the nucleolus stains deeply. The two nuclei are in some cases closely approximated or actually in contact, while in others they lie relatively remote from each other. The tip of each branch of the ascogenous hyphae forms a single definite hook (figs. 14–21). Although irregular hooks (figs. 17, 18) are not infrequent, complex systems of hooks such as those described by CLAUSSEN (**17**) in *Pyronema confluens*, by BROWN (**11, 12**) in *Leotia*, *Lachnea*, and *Geoglossum*, and by McCUBBIN (**53**) in *Helvella elastica* have not been found.

The two nuclei in the tip of the hypha at the time of crozier formation probably undergo conjugate division in the usual manner. Four nuclei (figs. 19–21) thus result. These drift apart, the uppermost pair passing into the bend of the hook, which then undergoes renewed growth and develops a prominent "dome cell" (fig. 21). The other pair of nuclei come to lie in such a position that one occupies the recurved tip of the hypha and the other the main body of the thread. The two septa frequently figured in

other Ascomycetes are then laid down, and the dome cell thus cut off develops into the ascus.

Ascus

The young ascus increases rapidly in size, and pushes upward among the paraphyses. It assumes a definite cylindrical shape, and its two nuclei, now closely approximated at its center, soon fuse (fig. 23). Fusion nuclei containing two nucleoli are frequently found (figs. 22, 23). After fusion the nucleus increases in size as the ascus enlarges. The two nucleoli evidently fuse, the fusion nucleolus being larger and staining deeply.

The chromatic material undergoes certain changes which call for special comment. The extrusion of chromatic bodies from the nucleus during synapsis or at early stages in meiosis is described by DIGBY (**23**) in *Galtonia candicans*, and by CARRUTHERS (**14**) in *Helvella crispa*. They state that these bodies may arise either from the nucleolus or nuclear framework. In both cases they are impregnated with chromatin. They are ejected forcibly through the nuclear membrane, and on escaping become definitely pyriform by constriction. They are sometimes drawn out behind into a fine thread and by means of this remain attached to the nucleus for a considerable time. Figures of these bodies given by CARRUTHERS resemble very closely similar bodies present in *R. undulata*. A comparison of the figures presented in the two cases shows them to be strikingly similar. However, the writer is unprepared to state that in *R. undulata* they actually represent ejected chromatin. It is certain that bodies taking the stain in a similar manner may be found in the cytoplasm of the ascus remote from the nucleus (figs. 22, 24, 27).

The mature ascus of *R. undulata* contains 8 unicellular hyaline spores. No attempt has been made to study the method of cutting out of the spores, nor has any critical examination been given to the nuclear divisions in the ascus.

General considerations

It is not necessary to review here the history of the development of our knowledge of the sexuality of the Ascomycetes. This task has been thoroughly accomplished by other workers. The earlier

papers bearing upon the subject are excellently reviewed by HARPER (40, 41), LOTSY (50), OVERTON (56), and GUILLIERMOND (37); while more recent literature has been discussed by FRASER (29), RAMSBOTTOM (58, 59), DODGE (25), and ATKINSON (1). It will prove profitable, however, to call attention to the more important general problems which are encountered in the investigation of the sexual phenomena in this group, and to review briefly the results of certain researches which bear directly upon our own study of *Rhizina undulata*.

The great difference of opinion which exists in the interpretation of the nuclear phenomena in the ascogonium, ascogenous hyphae, and asci has resulted in general uncertainty as to the real essence of sexuality in the Ascomycetes. Certain investigators maintain that the fusion nucleus of the ascus is the product of two successive nuclear fusions, the first of these taking place usually in the archicarp and constituting the sexual fusion, while the second occurs in the young ascus and is regarded as vegetative. HARPER (41) explains the occurrence of this second fusion in the ascus as an attempt on the part of the fungus to maintain the nucleocytoplasmic relation or equilibrium in the cell, a large cell such as the ascus requiring a large nucleus (DANGEARD 19, HARPER 38, WINGE 67). He states further that his researches indicate "that the fusion of the nuclei in the young ascus does not result in doubling the number of chromosomes as they appear in the succeeding divisions." Other investigators of this group, however, maintain that the fusion nucleus of the ascus is as the result of the two fusions necessarily tetraploid, and undergoes during the progress of the three divisions in the ascus a double reduction, the haploid number of univalent chromosomes being reached in each of the 8 resulting nuclei. FRASER (28 *Humaria rutilans*) and others (FRASER and BROOKS 31 *Humaria granulata*, *Ascobolus furfuraceus*, *Lachnea stercorea*, FRASER and WELSFORD 32 *Otidea aurantia*, *Peziza vesiculosa*, and CARRUTHERS 14 *Helvella crispa*) state also that the third division in the ascus accomplishes the second reduction by a unique process termed brachymeiosis. In the later stages of this mitosis, according to their accounts, whole chromosomes are pulled toward the poles, the number in the telophase thus being reduced to one-half that in the prophase.

Many other investigators, however, maintain that the nuclear fusion in the ascus constitutes the only fusion in the life cycle, and state that the third division in the ascus is a typical vegetative mitosis. FAULL (**26** *Hydnobolites*, *Neotiella*) and CLAUSSEN (**17** *Pyronema confluens*) state that the same number of chromosomes is found in each of the three divisions in the ascus, and HARPER (**40** *Pyronema confluens*, **41** *Phyllactinia Corylea*), who describes a double fusion, also finds the chromosome number remaining constant.

Although many Ascomycetes have been examined in the endeavor to reach a satisfactory solution of the questions involved in this controversy, investigators are now as far as ever from agreement. The minute size of the sexual nuclei and the consequent difficulty encountered in demonstrating fusion renders misinterpretation easy. It is possible, as suggested by BROWN (**12**), that nuclear division in the ascogonium has been mistaken for fusion. Moreover, the presence of V-shaped chromosomes in the third division in the ascus in some species at least probably explains the differences in chromosome counts made by different investigators. It is possible also that coalescence of degenerating nuclei has been mistaken for a sexual fusion.

It will be admitted also that two lines of a priori argument have contributed to the general disagreement concerning the essential facts in the nuclear history of the Ascomycetes. One group of investigators maintains that two successive nuclear fusions in a single life cycle, resulting in the production of a fusion nucleus with the $4x$ chromosome number, followed by a double reduction embracing the remarkable process of brachymeiosis, constitute a phenomenon so unusual as to warrant skepticism and to demand absolute proof. Since no similar variation has been found in any other group of organisms they doubt its occurrence in the Ascomycetes.

The other school of workers lay great stress upon the presence of 8 spores in the ascus of so many Ascomycetes, and point out that even in asci containing fewer spores than 8 the production of 8 nuclei as the result of the triple division of the fusion nucleus has been described in practically every species investigated. This almost universal occurrence of the triple division in the ascus is ascribed

by them as due to the "quadrivalent character" of the chromosomes in the fusion nucleus, which renders 3 mitoses necessary for the return to the univalent condition. When fewer than 8 spores are formed, the supernumerary nuclei degenerate (HARPER 41 *Phyllactinia Corylea*) or two or more nuclei are incorporated in one spore (WOLF 68 *Podospora anserina*). When many-spored asci are formed, additional vegetative nuclear divisions take place following the triple division.

In *Eremascus fertilis* (STOPPEL 60, GUILLIERMOND 36) the triple division occurs, but, as ATKINSON (1) has pointed out, there is here certainly only a single fusion, the antheridium and ascogonium being uninucleate and the fertilized ascogonium functioning as the ascus after fusion has occurred. Also in *Dipodascus albidus* (JUEL 47) and *Endomyces Magnusii* (GUILLIERMOND 36) essentially the same process takes place; a single nuclear fusion precedes spore formation, and the fertilized ascogonium functions directly as the ascus. In *Endomyces Magnusii*, moreover, according to GUILLIERMOND, only two divisions occur in the ascus and 4 uninucleate spores are formed.

The triple division in the ascus resembles very closely the process in the Basidiomycetes by which the basidium in some species (FRIES 33 *Nidularia pisiformis*, LEVINE 49 *Boletus* spp., *Strobilomyces strobilaceus*) produces as the result of 3 successive nuclear divisions 8 nuclei, which appear in 4 binucleate spores. Since in these cases the 3 divisions follow one another rapidly and a rest period then ensues, the resemblance to the process in the Ascomycetes is marked. LEVINE describes the third division as taking place always in the spore, and states that in *Boletus albellus* a fourth division occurs, the resulting 4 spores being tetranucleate. FRIES states that in *Nidularia pisiformis* uninucleate spores are never found, and says that immediately upon the entrance of the nucleus completely into the spore a spindle is seen forming. He believes that the nucleus while migrating through the canal of the sterigma is already in the prophase of division. When it reaches the spore the equatorial plate is formed at once. MAIRE (51) in *Clavaria rugosa* and *Cantharellus cinereus* figures the third division as taking place in the basidium itself.

The questions involved in the study of the nuclear history of the Ascomycetes will never be satisfactorily answered by a priori argument. The careful examination of a large number of representatives of this group presenting peculiarly favorable material for investigation, and the comparison of the data obtained with those available for other groups will, however, go far toward explaining the discrepancies in conflicting accounts and toward answering vexing questions to the satisfaction of all students.

The greatest variation is evident in the morphology of the sexual apparatus in the Ascomycetes even in forms in which the gross structural characters of the ascocarp are very similar. The published evidence would seem to show, moreover, that a certain amount of variation in the unfolding of the sexual phenomena may be encountered in the investigation of even a single species.

In *Pyronema confluens* the sexual phenomena have been variously described. HARPER (40) gives in detail the passage of the male nuclei from the antheridium into the ascogonium, their fusion there in pairs with the female nuclei, the migration of the fusion nuclei into the ascogenous hyphae, and later a second fusion in the ascus. CLAUSSEN (16, 17) also describes the entrance of the antheridial nuclei into the ascogonium, but states that they merely pair there with the female nuclei without fusion. These pairs of nuclei then migrate into the ascogenous hyphae where they divide conjugately, two nuclei ultimately fusing in the ascus to give a fusion nucleus with the diploid number of chromosomes. This demonstration by CLAUSSEN of conjugate divisions in the ascogenous hyphae is especially noteworthy, since these divisions in the undifferentiated portions of the hyphae have not been demonstrated elsewhere in the Discomycetes. Since these nuclei divide conjugately, there is good reason to feel that they are linked together by a sexual attraction. FRASER (30), however, says that "the phenomenon of conjugate division is probably but a special example of the very general fact that nuclei present in the same cell usually divide simultaneously" (FROMME 34, OLIVE 55). WELSFORD (66) suggests that the paired condition of the nuclei may be merely the response to the physiological conditions usually found in rapidly developing hyphae. VAN TIEGHEM (62) grew under cultural con-

ditions a form which he stated to be *Pyronema confluens* and was able to develop normal or rudimentary antheridia or to suppress them entirely, while the ascogonia developed normally under all conditions. DANGEARD (22) found in what he regarded as the same species that even in cases in which the antheridium fuses with the trichogyne the male nuclei degenerate *in situ*, and fail to enter the ascogonium. BROWN (10, 13), working with a strain which he has named *Pyronema confluens* var. *inigneum*, found that the ascogonia and antheridia fail to fuse, and states that only one nuclear fusion, that in the ascus, occurs in the life cycle. He also examined the parent species, and states that in it he found migration of male nuclei into the ascogonium. *Pyronema confluens* var. *inigneum*, according to the account of BROWN, differs from the parent species physiologically also in that it grows freely upon an unsterilized substratum. The variation in the accounts of the different workers who have examined this species would seem to show that in this form the degeneration of the antheridium is now taking place. On account of the small size of the nuclei the demonstration of fusion in the ascogonium, however, is extremely difficult and it is possible that two investigators might reach a different conclusion from the examination of a single set of slides.

The writer feels that neither in *Pyronema confluens* nor in any other Ascomycete have two successive nuclear fusions in a single life cycle been conclusively demonstrated. It is evident that we cannot depend upon a critical examination of the nuclear divisions in the ascus to tell whether or not one or two fusions have occurred, since here also a fundamental difference in interpretation exists. Although FRASER and her co-workers figure and describe brachymeiosis in several species, HARPER and others find the chromosome number remaining constant throughout the three divisions in the ascus.

Summary

1. The sexual process has not heretofore been studied in any member of the Rhizinaceae. The examination of *Rhizina undulata* Fries is therefore of considerable interest.

2. Material for study was collected at Ithaca, New York, and a paper describing the origin of the apothecium in this species has already been published (**27**).

3. The vegetative mycelium is parasitic on the roots of trees, and develops profusely in the soil. On the surface of the ground or on parasitized roots minute primordia of fruit-bodies are developed. These are composed of undifferentiated hyphae which form at the periphery a somewhat indefinite palisade layer.

4. After the ascocarp primordium has attained a diameter of approximately 1 mm., certain hyphae near its center are transformed into archicarps. As many as 8 archicarps may be developed in a single ascocarp.

5. The individual archicarp develops by the rapid growth and transformation of an ordinary multicellular hypha. Its cells are multinucleate from the first. The nuclei increase greatly in number by repeated division and the archicarp soon takes on a dense opaque appearance.

6. An antheridium is absent.

7. The archicarp develops in some cases as a loose coil, and in others winds irregularly among the other hyphae, but tight coils have not been found. The number of cells in a single archicarp has been found to vary from 10 to 19 or more.

8. The terminal cell of the archicarp is small and attenuated, and at maturity shows disorganized protoplasmic contents. It has been here from analogy termed the trichogyne, but it certainly does not function.

9. As the archicarp approaches maturity a single, very prominent, deeply staining, hemispherical or convex pad appears on each side of the transverse septa. These pairs of deeply staining pads apparently represent a swelling of the wall due to gelatinization at that point. They later fuse and finally disappear, leaving a large pore in the septum.

10. Approximately one-half of the cells of the archicarp lying at the center of the coil now put out ascogenous hyphae. The remaining basal and apical cells fail to bud, and their nuclei and cytoplasm flow through the pores in the transverse septa into the ascogonial cells, and thence into the ascogenous hyphae.

11. With the outward flow of nuclei and cytoplasm into the ascogenous hyphae, the cytoplasm in the ascogonial cells of the archicarp becomes pronouncedly vacuolated. The nuclei are then seen to lie in pairs. In preceding stages the dense nature of the protoplasm and the crowding of the nuclei render the demonstration of a paired condition extremely difficult. Pairs of nuclei in the archicarp have been seen only in cells giving rise to ascogenous hyphae.

12. Careful search has failed to demonstrate stages of nuclear fusion in the ascogonial cells or in the ascogenous hyphae.

13. Paired nuclei are also present in the ascogenous hyphae. Neither conjugate nor simple divisions have been demonstrated.

14. Crozier formation takes place, but elaborate systems of hooks at the ends of the ascogenous hyphae have not been found. Nuclear fusion occurs in the young ascus.

DEPARTMENT OF PLANT PATHOLOGY
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EXPLANATION OF PLATES III AND IV

All the figures were drawn with the aid of an Abbé camera lucida, and various combinations of lenses were used. The drawings have been reduced two-sevenths in reproduction. Figs. 1–4 were built up from consecutive sections of a series, two sections each being used for figs. 1 and 2, and three sections each for figs. 3 and 4. This was necessitated by the fact that the archicarp rarely lies for any considerable portion of its length in the plane of one section.

Strictly speaking, however, these drawings are not composite, since the individual cells were outlined as they appear in a single section. In fig. 2 the terminal 6 cells were outlined from one section, and the basal 3 from the adjacent section. All of the central portion of the archicarp shown in fig. 3 was outlined from one section, although portions of these cells appear in the two adjacent sections. This explains the presence in the drawings of the deeply staining pads or open protoplasmic connections at certain septa and their absence at others where they lie outside the plane of the optical section. In those cases in which they are not shown, the examination of other optical sections usually shows either a pad or a pore, but in some cases they are obscured by the dense overlying protoplasm of one or the other of the adjacent cells. In fig. 4, due to the absence of winding in the archicarp, many of the open protoplasmic connections appear in one plane. In drawing the terminal cell of the archicarp shown in fig. 3 an exception has been made to the general method of treatment. This cell on account of its coiled nature cannot be shown satisfactorily in a single plane, but since it lies wholly in one section it has been possible to draw it in perspective. The nuclei shown in figs. 1–4 have not been outlined with the camera lucida, and the writer has attempted to show in the cells of these archicarps merely the relative number and size of the nuclei, not their exact position. The dense nature of the cytoplasm at these stages, the crowding of the nuclei, and the use of several optical sections in the preparation of the drawings renders a faithful portrayal of the nuclei impossible. The remainder of the drawings (figs. 5–27) have been made from a single optical section and the nuclei and other cell contents are accurately reproduced.

PLATE III

FIG. 1.—Terminal portion of young archicarp of *Rhizina undulata* Fries, ×500; note dense protoplasmic contents, numerous nuclei, and deeply staining convex pads on transverse septa of lower and more nearly mature cells; the fact that the terminal cells are long and slender and contain relatively few nuclei indicates the origin of the archicarp from a vegetative hypha.

FIG. 2.—Terminal portion of a somewhat older archicarp, ×500; note attenuated terminal cell, trichogyne; deeply staining pads and open protoplasmic connections cannot be seen on all septa since they lie outside plane of section.

FIG. 3.—An entire archicarp nearing maturity, ×500; 2 ascogonial cells have already begun to put out ascogenous hyphae; deeply staining pads are prominent on several septa; open protoplasmic connections have resulted from their disappearance on others.

FIG. 4.—An entire archicarp contrasted in size with ordinary hyphae of ascocarp, ×500; here open protoplasmic connections are visible at practically every septum.

FIG. 5.—Cells in basal region of archicarp at time of general flow of nuclei and cytoplasm into ascogonial cells, ×500.

FIG. 6.—Cells in apical region of another archicarp when this phenomenon is taking place, ×500.

FIG. 7.—Ascogonial cells of archicarp putting out ascogenous hyphae, ×500; note paired nuclei, and persistence of a single pair of deeply staining pads.

PLATE IV

FIGS. 8, 9.—Transverse sections through budding ascogonial cells at points between places where ascogenous hyphae arise, ×1315; note vacuolated cytoplasm and paired nuclei; no fusion stages have been observed; in those cases in which a solitary nucleus appears, its companion lies either above or below.

FIGS. 10, 11.—Transverse sections through budding ascogonial cells, ×1315; in these cases ascogenous hyphae at their point of origin lie in plane of section; note paired condition of nuclei in ascogenous hyphae.

FIG. 12.—Ascogenous hyphae midway between ascogonial cells and developing hymenium, ×1315.

FIG. 13.—Terminal branches of ascogneous hypha just preceding crozier formation, ×1315.

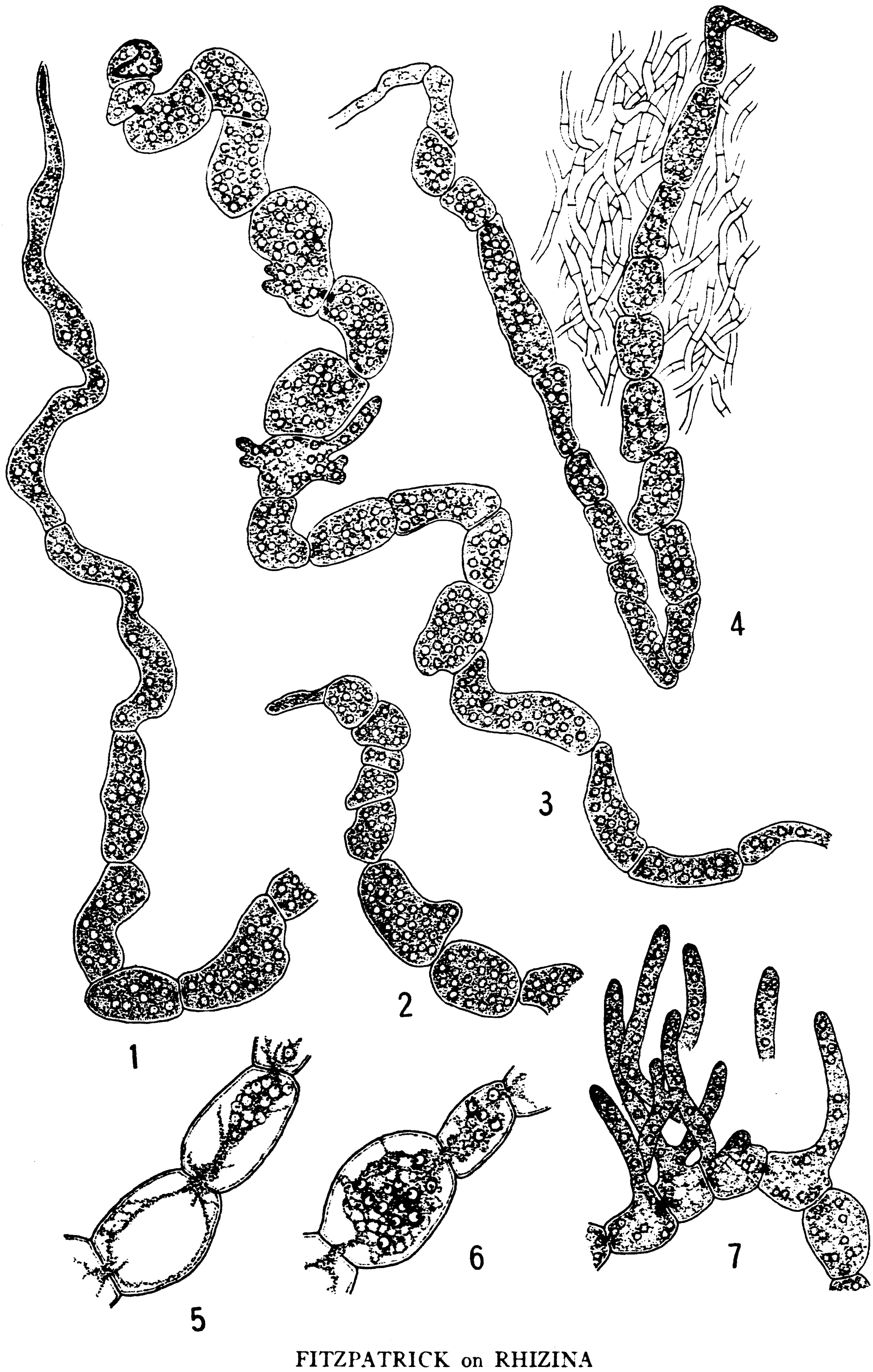
FIGS. 14–18.—Ascus hooks containing single pair of nuclei preceding conjugate division, ×1315.

FIGS. 19–21.—Ascus hooks containing 4 nuclei after conjugate division has taken place, ×1315.

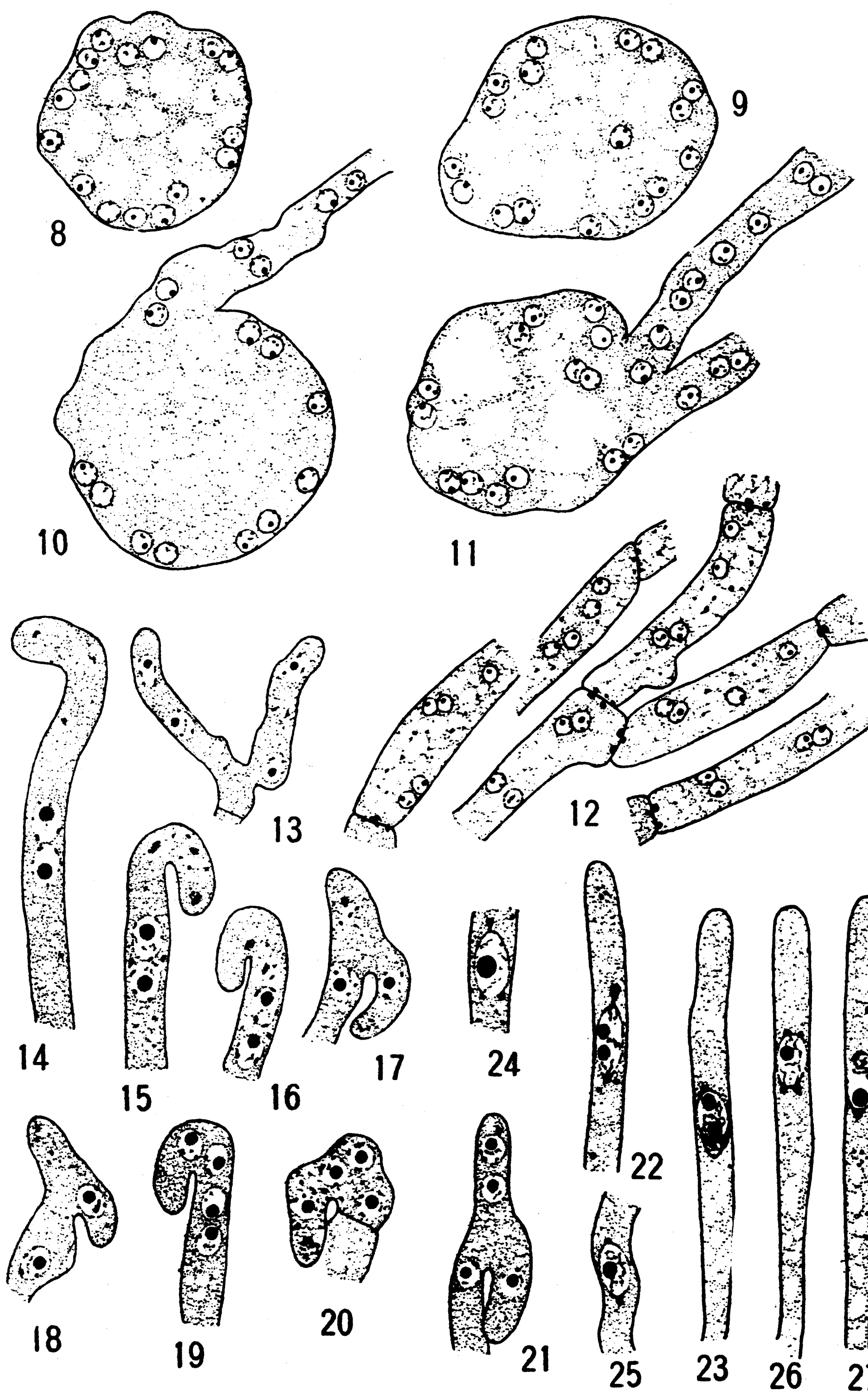
FIGS. 22, 23.—Young asci, fusion nucleus in each still showing two nucleoli, ×830.

FIG. 24.—Fusion nucleus containing single fusion nucleolus, ×1315.

FIGS. 25–27.—Young asci showing fusion nucleus with single nucleolus, ×830.



FITZPATRICK on RHIZINA



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